# EGG TRANSFER IN CATTLE

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## SUMMARY

The techniques of superovulation of donor cows, egg recovery and transfer used in a large-scale egg transfer programme are described together with details of management of a large herd of recipient cows. Slightly more than 75% of eggs were recovered and of these approximately 80% were fertilized. During the latter part of the first season a mean of 1.9 pregnancies per donor operation was achieved in comparison with 3.7 pregnancies per donor operation in the second season. Cows treated with PMSG for a second or third time had a lower ovarian response than cows treated for the first time. There was a small decline in ovarian response and in the mean number of pregnancies per donor operation in the autumn-winter period in comparison with results from the spring-summer period.

## INTRODUCTION

VARIOUS GROUPS, mainly in North America, U.K. and Australia, have used egg transfer as a means of multiplying "exotic" cattle since 1972. However, there are few available data on the success of these ventures. Early in 1973, "Southern Breeders", a group of cattle producers, became interested in the technique and invited the writers to carry out this work.

This paper summarizes the results of operations in which eggs were transferred into 691 recipient cows. Methods described are currently being used as the programme continues.

## EXPERIMENTAL

Prior to July 28, 1973, the date of the first operation on an "exotic" cow, 20 Hereford cows were superovulated and eggs were recovered in order that the skills of superovulation, surgery and egg recovery would become familiar. From July 1973 until August 1974 (Season 1), 56 donor operations were carried out and also a further 62 operations between November 1974 and June 1975 (Season 2).

TABLE 1: SEQUENCE OF SUPEROVULATION, SYNCHRONIZATION AND INSEMINATION OF DONOR COWS

| *Day Cycle* | *Period in Day* | *Treatment* |
|---|---|---|
| 0 (oestrus) | | |
| 11 | a.m. | PMSG 1500-2000 i.u. |
| 13 | p.m. | PG $F_2\alpha$ 20 mg |
| 14 | a.m. | PG $F_2\alpha$ 10 mg |
| 15 (oestrus) | | |
| 15 | p.m. | 1st insemination |
| 16 | a.m. | 2nd insemination |
| 16 | p.m. | 3rd insemination[1] |

[1] Only if the donor cow is in oestrus longer than 12 hours.

## Synchronization and Superovulation of Donor Cows

The sequence of events associated with treatment of donor cows and insemination is shown in Table 1. Prior to treatment, all cows were in good physical condition, were cycling normally, and in most cases had had at least two consecutive heats. Donor cows injected with pregnant mare's serum gonadotrophin (PMSG) and prostaglandin $F_2\alpha$ were almost invariably in oestrus 24 hours after the second injection of prostaglandin. The dose of PMSG was calculated according to body weight, age, condition of the prospective donor and previous experience. Consequently, a strict dose-bodyweight relationship was not always followed.

Two straws of "commercial" semen were usually used. Cows were inseminated only three times when they were in oestrus for longer than 12 hours. The operation on the donor was carried out on days 5, 6 or 7 after insemination. The start of day 1 was taken as 6 hours after the end of standing heat. During the first season some operations were done on day 7; as the results were very poor subsequent operations were done on days 5 or 6, with better results.

## Recovery and Classification of Eggs

All donor and recipient cows were deprived of feed and water for 48 hours before surgery. An intramuscular injection of 20 mg of acetylpromazine was given approximately 15 minutes before the intravenous administration of thiopentone sodium as a pre-anaesthetic. In the operating theatre the animal was intubated and closed circuit anaesthesia (halogen/oxygen mixture) administered.

Cows were placed in dorsal recumbancy and the operating area prepared and draped. An incision 15 cm long was made in the mid-line just anterior to the udder. After the uterus and ovaries were exteriorized, the number of corpora lutea were counted. Extreme care was taken to avoid bleeding and damage to the ovaries and uterus which were washed with heparinized saline and kept moist throughout the operation.

A cuffed flexible catheter was inserted into the lumen of the uterine horn just anterior to the uterine bifurcation. Two methods of flushing were used: between 50 and 100 ml of flushing medium was introduced either through a blunted 18 gauge needle inserted into the tip of the horn or via a tomcat catheter inserted into the Fallopian tube. The first method was used on day 6 or when undue tension was required to insert the catheter into the Fallopian tube, thus alleviating possible damage to the fimbria. The second method was used on day 5 when it was easier to exteriorize the fimbria and minimize handling. The flushing procedure was repeated for each uterine horn.

The flushings were collected in 15 ml glass bowls and immediately transferred to an incubator at 35° C. Siliconized glassware and solutions were warmed to 35° C prior to the operation. The flushing medium was TCM 199 Hepes, buffered (7.2 to 7.4) to which inactivated foetal bovine serum had been added to a final concentration of 10%.

After recovery of eggs the reproductive tract was again washed with heparinized saline and returned to the abdomen. The peritoneum and linea alba (No. 10 E.P. Chromic), and fat (No. 9 E.P. Chromic) were sutured with a single running suture. Horizontal mattress sutures with braided nylon were used in the skin. The cow was then removed from anaesthesia but the endotracheal tube was not removed until a swallowing reflex returned. Cows were propped up in a sitting position with straw bales in a recovery area adjacent to the surgery. Both donor and recipient cows were given 5 million units of penicillin and dihydrostreptomycin daily for three days after surgery. Following surgery donor cows were examined rectally every 2 or 3 days for evidence of palpable adhesions. These were broken down by gentle manipulation through the rectal wall.

Eggs observed in the bowls with the aid of a dissecting microscope were classified on the basis of a morphological examination indicating fertilization and whether or not development had proceeded to the expected stage — *i.e.*, day 5 recovery, 16 to 32 cells; day 6 recovery, 32 to 64 cells.

The classification of fertilized eggs was:

A. Fully developed.
B. Fully developed but with uneven cell development, or appearing "normal" but one stage of cell division less than expected.
C. More retarded than B eggs or with some obvious cellular abnormality.

### Transfer of Eggs

Where possible recipient cows were at exactly the same stage of the oestrous cycle as the donors. When insufficient exactly synchronized cows were available the first preference was given to animals whose previous onset of oestrus was within 12 hours of the donor. Only rarely were recipients less well synchronized (max. ± 24 h). Pretreatment and exteriorization of the reproductive tract was identical with that described for donor cows. Eggs were transferred to the uterine horn adjacent to the ovary with the corpus luteum. A small hole was made into the uterine lumen using a blunt probe and eggs transferred with approximately 0.1 ml of flushing fluid via a fine pasteur pipette passed towards the body of the uterus. Recipients were pregnancy tested 60 to 90 days after transfer. Most recipients received only one egg. However, in 31 cases two eggs were transferred, owing to the limited number of recipients, or to B and/or C eggs being transferred together.

### Management of the Recipient Herd

Efficient and painstaking management of the recipient herd is critical in the success of any egg transfer programme. In the present programme 500 to 600 recipient cows are run with teaser bulls (usually one bull per 50 cows). All bulls have a redirected penis which eliminates any chance of transmission of venereal disease. Oestrus is recorded at least twice daily and appropriate cows drafted out when donor operations are due. In most cases this has ensured 15 to 20 animals in oestrus at exactly the same time as a donor cow scheduled for surgery.

Recipient cows must be in good condition and preferably gaining weight at the time of surgery. For the average donor operation it is estimated that 60 man-hours are required for oestrus detection, recording, drafting of cows, surgical recovery of eggs and transfer to recipients, as well as post-operative treatment of animals.

Animals used as recipients were adult Herefords, 2- and 3-year-old Hereford × Angus, Friesian and Friesian-cross cows. Parity and breed of recipients appear to have no effect on the success rate of transfers.

### Recording

Accurate recording at all stages is necessary for the efficient running of the programme. All donors and calves resulting from egg transfer are blood typed at the University of Queensland. Blood samples are collected when calves are more than 30 days of age. Following parentage determinations, certificates are issued for each calf, enabling registration with appropriate breed societies.

## RESULTS AND DISCUSSION

### Operations during the First and Second Seasons

The egg recovery rate during this work has been very satisfactory with more than 75% of eggs shed being recovered from the uterus. The percentages of Type A, B, C and unfertilized eggs recovered in all of the donor operations were 68.9, 6.6, 4.6 and 19.9, respectively.

During the first 30 donor operations in the first season, the numbers of corpora lutea on the ovaries were not recorded. One hundred and seven fertilized eggs (mean of 3.6 per donor) were transferred, which resulted in 43 pregnancies, an average of 1.4 pregnancies per donor operation. Results from the remaining 26 donor operations in the first season and all operations in the second season are presented in Table 2. Pregnancy data presented refer to positive pregnancy diagnoses 90 days after transfer. A much higher pregnancy rate per donor operation was achieved

TABLE 2: DONOR PERFORMANCE AND RESULTS OF TRANSFERS DURING TWO SEASONS

| | *Season 1* | *Season 2* |
|---|---|---|
| No. of donors .... .... .... .... .... .... | 26 | 62 |
| Corpora lutea .... .... .... .... .... .... | 9.8 | 12.3 |
| Eggs recovered per donor: | | |
| Mean number .... .... .... .... .... | 7.5 | 9.5 |
| Percent .... .... .... .... .... .... | 76.5 | 77.2 |
| Eggs transferred .... .... .... .... .... .... | 6.7 | 7.2 |
| % Pregnancies per eggs transferred .... .... .... | 29.9 | 54.8 |
| Pregnancies per donor operation .... .... .... | 1.9 | 3.7 |

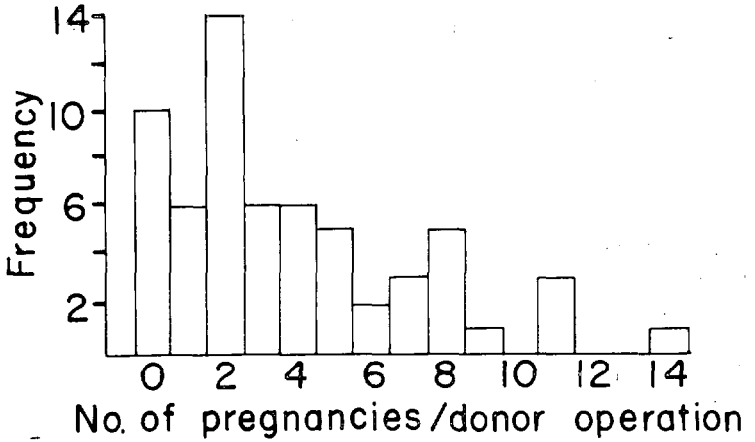


FIG. 1: *Frequency distribution of number of pregnancies per donor operation from cows treated during the second season.*

in the second season. This was mainly due to a higher percentage of pregnancies per eggs transferred, although the difference in ovulation rates also contributed. This greater success was also due to operating on donors on days 5 or 6 and not on day 7 and to the greater experience in the transfer technique. In addition, the use of a more reliable flushing medium and greater experience in management of the recipient herd probably had some effect. The data in Table 2 may be compared with those presented in a comprehensive review of Gordon (1975) where the mean number of corpora lutea was 18.1, with a mean of 9.8 being recovered and only 4.3 being considered fertilized.

TABLE 3: DONOR PERFORMANCE AND RESULTS OF TRANSFERS FROM COWS OPERATED FOR THE FIRST, SECOND AND THIRD TIME DURING THE SECOND SEASON

(Some animals operated on for the first and second time during the first season)

| | *Donor Operation* | | |
|---|---|---|---|
| | *1st* | *2nd* | *3rd* |
| No. of donors .... .... .... .... .... | 34 | 23 | 5 |
| Corpora lutea .... .... .... .... .... | 15.1 | 8.5 | 10.8 |
| Eggs recovered per donor: | | | |
| Mean number .... .... .... .... | 11.2 | 7.0 | 10.0 |
| Percent .... .... .... .... .... | 74.2 | 82.4 | 92.6 |
| Eggs transferred .... .... .... .... | 8.1 | 5.9 | 7.8 |
| % Pregnancies per eggs transferred .... .... | 54.3 | 57.0 | 50.0 |
| Pregnancies per donor operation .... .... | 4.3 | 3.0 | 3.2 |

The number of pregnancies per donor operation is shown in Fig. 1. Ten of 62 operations did not result in a pregnancy and the highest number of pregnancies from a single operation was 14.

## Results after Several Operations

Of the 62 operations during the second season, 34, 23 and 5 were the donors first, second and third operations, respectively (Table 3). Some of the cows were operated on for the first and second time during the first season. The ovulation rate was lower following the second and third treatments but the recovery of eggs, fertilization rates and the percentage of pregnancies per eggs transferred was not affected.

TABLE 4: DONOR PERFORMANCE AND RESULTS OF TRANSFERS FOM EIGHT COWS OPERATED FOR THE FIRST AND SECOND TIME DURING THE SECOND SEASON

| | *Donor Operation* | |
|---|---|---|
| | *1st* | *2nd* |
| No. of donors .... .... .... .... .... .... | 8 | 8 |
| Corpora lutea .... .... .... .... .... .... | 15.8 | 8.4 |
| Eggs recovered per donor: | | |
| Mean number .... .... .... .... .... | 12.6 | 6.5 |
| Percent .... .... .... .... .... .... | 79.7 | 77.4 |
| Eggs transferred .... .... .... .... .... .... | 9.1 | 5.9 |
| % Pregnancies per eggs transferred .... .... .... | 64.8 | 52.5 |
| Pregnancies per donor operation .... .... .... | 5.9 | 3.1 |

Data from eight cows operated twice within the second season are in Table 4. All but one cow had both operations in either the November to March or the April to June period. The dosages of PMSG used at the two times varied slightly (within animals not more than 300 i.u.) and the mean dose for the first and second operations was 1788 i.u. and 1862 i.u. PMSG, respectively. Following treatment with PMSG the second time, the mean number of ovulations was reduced ($P < 0.05$) which also reduced the mean number of pregnancies per donor operation. These data are consistent with the decline in ovarian response to repeated doses of PMSG reported by Willet *et al.* (1953) although at variance with other reports (see review by Gordon, 1975).

TABLE 5: DONOR PERFORMANCE AND RESULTS OF TRANSFERS FROM COWS OPERATED DURING NOVEMBER TO MARCH, AND APRIL TO JUNE DURING THE SECOND SEASON

| | *Months* Nov.-Mar. | Apr.-Jun. |
|---|---|---|
| No. of donors .... .... .... .... .... .... | 39 | 23 |
| Corpora lutea .... .... .... .... .... .... | 13.2 | 10.4 |
| Eggs recovered per donor: | | |
| Mean number .... .... .... .... .... | 10.3 | 8.0 |
| Percent .... .... .... .... .... .... | 78.0 | 76.9 |
| Eggs transferred .... .... .... .... .... | 8.2 | 5.4 |
| % Pregnancies per eggs transferred .... .... .... | 58.3 | 46.3 |
| Pregnancies per donor operation .... .... .... | 4.4 | 2.5 |

## Effect of Time of the Year

During the second season ovulation rates and the percentage of pregnancies per eggs transferred dropped slightly, resulting in a reduction in the mean number of pregnancies per donor operation (Table 5). A seasonal variation in superovulatory response has also been reported by Scanlon (1969) who recorded a decline in response in the autumn-winter period. Climatic conditions and therefore the availability of feed make it difficult to keep recipient cows in good condition as winter approaches. This may be involved in the lower percentage of pregnancies per eggs transferred.

## CONCLUSIONS

Several factors contribute to the success or otherwise of a commercial egg transfer programme.

A reliable technique to achieve superovulation must be developed as this aspect of the work is the most unpredictable at present. The amounts of PMSG used were aimed at producing 15 to 20 ovulations per donor. Considerable practice is also necessary to develop the surgical skills which are essential for high egg recovery rates and also to minimize damage to the reproductive tract and adhesions which may result.

The management of the recipient herd is vital to the success of the undertaking. Accurate heat detection, good husbandry and nutrition are required to attain the objectives.

Provided the superovulatory and surgical techniques are satisfactory in conjunction with a high level of competence in recording and management of donors and recipients, a successful egg transfer programme should result. However, although the ovarian

response to PMSG is variable and the success rate of transfers from individual animals is also variable, the mean number of pregnancies per donor operation represented a considerable increase in the number of progeny obtained compared with natural breeding.

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